DOI: 10.21451/1809-3000.RBRA2025.019

Anais do XXVI Congresso Brasileiro de Reprodução Animal
Curitiba, PR, 07 a 09 de maio de 2025.

ARTs for Zoo Population Management

Reproductive biotechnologies in zoo population management

Gabriela F. Mastromonaco[1]* (https://orcid.org/0000-0001-6229-0355) and Marcia de Almeida Monteiro Melo Ferraz[1] (https://orcid.org/0000-0002-9896-3459)

[1] Reproductive Sciences Unit, Toronto Zoo, 361A Old Finch Avenue, Toronto, M1B 5K7, ON, Canada
Reproductive Sciences Unit, Toronto Zoo, 361A Old Finch Avenue, Toronto, M1B 5K7, ON, Canada

Abstract

Global biodiversity continues to decline at an alarming rate, with increasing numbers of species at risk of extinction due to climate change, habitat loss and other human activities. In response, conservation efforts are shifting toward integrated population management strategies that bridge in situ and ex situ environments. Historically, zoos focused on managing small, closed populations, which proved insufficient for maintaining long-term genetic viability. Today, there is growing recognition of the need to incorporate genetic diversity from wild populations, utilize biobanking to preserve genetic resources, and apply assisted reproductive technologies (ARTs) to enhance species recovery. This review examines the evolution of zoo-based population management, emphasizing the importance of genetic tools and cooperative breeding strategies. We explore the development and application of ARTs—artificial insemination, in vitro embryo production, embryo transfer, cloning, and stem cell technologies—and highlight both their conservation potential and barriers to broader implementation, including biological, logistical, and regulatory constraints. By compiling current knowledge, challenges, and examples across taxa, this review provides a practical and conceptual foundation for advancing the use of ARTs and biobanking in species conservation.

Keywords: *Biobanking, genetic management, zoo conservation, in situ–ex situ integration*

Introduction

Communities around the world continue to struggle with the on-going impacts of human activity that are disrupting ecosystem functions and ultimately driving the detrimental changes in climate and biodiversity to critical levels. The World Wildlife Fund's Living Planet Report 2024 (1) highlighted a 73% decline in monitored species over the past 50 years. Similarly, the Stockholm Resilience Centre's 2023 study of 9 planetary thresholds, such as ocean acidification, indicated that we are poised to cross the planetary tipping point (2). These data support the need for global action as laid out in the Convention on Biological Diversity's Kunming-Montreal Global Biodiversity Framework (3). It highlights several key targets for 2030 with Target 4 (halt species extinction and protect genetic diversity) focused specifically on pausing and reversing biodiversity loss. Preserving biodiversity requires mitigating the causative factors: human behaviour, natural resource extraction, land use, and ultimately, climate change. The importance of insurance populations in managed care in zoos, aquariums and botanic gardens has become critical to supporting species at risk in the wild. This led the International Union for the Conservation of Nature Species Survival Commission (IUCN SSC) to recognize the significant contributions of *ex situ* populations under human care to conserving animals, plants and fungi (4). Not only is the genetic management of living populations outside their natural habitats critical to species restoration efforts, but novel approaches are required to ensure long-term genetic and demographic health of threatened species. The aim of this paper is to provide an overview of *ex situ* population management strategies and the importance of implementing assisted reproductive technologies to support species sustainability goals.

Population Management for Conservation

The long-term survival of a species depends on populations that are genetically, demographically, and physiologically healthy, with the capacity to adapt and evolve in response to changing environmental conditions. Increased risk of extinction is correlated with loss of genetic diversity, including deleterious consequences from inbreeding and mutation accumulation [reviewed by (5)]. Small or isolated populations, a concern for both wild populations in highly transformed habitats and managed populations dispersed in zoos around the world, face a greater risk due to reduced genetic variability and increased vulnerability to

Correspondência: *gmastromonaco@torontozoo.ca
Recebido: 09 de abril de 2025
Aceito: 30 de abril de 2025

demographic and environmental stochasticity (6). Therefore, population management strategies for wildlife conservation focus on identifying the appropriate conservation unit (i.e., species, subspecies, or population) and preserving as much of its diversity as possible.

Zoo populations

Almost 40 years ago, Soulé and colleagues published a seminal paper that introduced the concept of the 'millennium ark,' emphasizing the critical role of breeding endangered species in zoos and botanic gardens to help prevent extinction (7). They hypothesized the number of species, number of individuals, and length of time in managed care needed to protect biodiversity while the natural environment is being restored. Most importantly, they proposed the “reasonable goal” of retaining 90% genetic variability of the founder individuals for 200 years, a framework so impactful that it transformed the way zoos manage animal populations and continues to shape practices to this day (albeit over a 100-year time frame). Zoos responded by establishing cooperative breeding programs (e.g., Species Survival Plans® in North America, *Ex Situ* Programmes in Europe) to avoid inbreeding thereby supporting the primary goal of maintaining genetic diversity. Within 20 years, however, the challenges of sustaining the ‘ark’ became clear, as evidenced by low numbers of founders and reproductive rates, and consequently, populations below target size (8). These factors, along with limited space and resources, are among the key determinants of long-term viability of zoo populations. Even in the early work by Soulé et al., the value of developing assisted reproductive technologies (ARTs) to support the genetic management of small populations was already being acknowledged (7).

Due to the consequences incurred from loss of genetic diversity, including susceptibility to disease and inability to respond to environmental stressors, *ex situ* programs prioritize genetic management of their animals. However, these programs are typically small, closed populations due to the constraints mentioned above. Demographic changes occur when there are insufficient individuals to compensate for decreased reproductive output (low fertility or survival), altered age distribution and skewed sex ratio (9). Similarly, a limited number of breeding individuals increases the risk of inbreeding, contributing to a reduction in genetic diversity that can be further amplified by random genetic drift. Notably, individuals more likely to breed may result in unintentional selection for traits suited to *ex situ* conditions, potentially leading to inadvertent domestication, an unwanted outcome when maintaining insurance populations for future reinforcement of the wild (10). In steelhead (*Oncorhynchus mykiss*), researchers found that a single generation in a managed environment was sufficient to select for traits advantageous in *ex situ* conditions but potentially detrimental in the wild (11). Wild-born fish that were most successful in the hatchery environment produced offspring with the poorest performance in the wild. Introducing founder genetics not only at the start of the program but at intervals throughout the program’s lifespan – either from within the *ex situ* population (i.e., under- or non-represented individuals) or brought in from the *in situ* population (i.e., novel individuals) – will help ensure the managed population remains representative of the wild gene pool over time (9).

To date, the most effective approach for genetic management in zoo populations is to ensure minimal relatedness (low mean kinship) between breeding individuals. An animal’s mean kinship identifies its average relatedness with all living members of the population. Establishing breeding plans based on lowest mean kinship between breeding pairs was determined to most likely capture rare alleles and equalize representation of founder genomes (12). This strategy depends on accurate record-keeping to establish individual pedigrees documented in regional or global studbooks, a process that requires collaborative precision, especially as animals, and increasingly, sperm and embryos, are exchanged between facilities to support low mean kinship breeding recommendations. A recent study in Cuvier’s gazelle (*Gazella cuvieri*) used pedigree records over 48 years to assess potential loss in genetic diversity in the current population (13). The *ex situ* program for this endangered gazelle species was established from 4 founders (1 male and 3 females), a high risk of inbreeding right from the start. The study revealed that, during the early years of the program, animals transferred from Europe to North America did not include descendants of one of the female founders. This omission led to population structuring, which may threaten the viability of the North American population and any herds derived from it (13). While some *ex situ* populations are unsustainable, Che-Castaldo et al. found that, in most of the 400 *ex situ* vertebrate programs they examined, there were no significant declines in genetic and demographic characteristics typically associated with small populations (14).

Advancements in genomics and the dramatic drop in genome sequencing costs, from millions of dollars in the early 2000s to just a few hundreds today, have given rise to new approaches in genetic management of endangered species. Incorporating genomic methods, particularly with the possibility of non-invasive sampling (e.g., feces and shed skin, feather or hair), serves two important purposes: evaluating the genetic integrity of the population and guiding breeding recommendations. Genomic data, including sequence, transcriptome, and genotyping information, will soon become essential tools for understanding individual and population genetic profiles that will enable managers to make informed decisions that maximize genetic diversity while minimizing unintended consequences (e.g., phenotypic or behavioural changes) (15). A recent study on the scimitar-horned oryx (*Oryx dammah*), extinct in the wild and bred in conservation programs for over 50 years, found higher genetic diversity than most mammals experiencing a small founder event, and attributed this outcome to successful management strategies (16). The work was inspired by the recent initiation of reintroduction efforts for the species, emphasizing the role for genetic assessments in the selection of founders for the wild population. In the pink pigeon (*Nesoenas mayeri*), researchers developed a novel bioinformatics pipeline to analyze genomic data from 6 breeding individuals, enabling the identification of optimal breeding pairs to reduce inbreeding depression and genetic load in their offspring (17). The combination of genetic assessment with ARTs offers a more comprehensive approach to addressing the long-term challenges of *ex situ* population management.

I.b Integrated populations

Although species management practices *in situ* (e.g., threat assessment, population monitoring) vary from those implemented *ex situ* (e.g., breeding, population planning), both aim to maintain healthy and resilient populations. In this regard, whether in natural or intensively managed settings, decreasing population sizes increase the risk of genetic unsustainability, and thus, probability of extinction. To enhance the achievement of long-term genetic targets for threatened species, the IUCN SSC's Conservation Planning Specialist Group began promoting integrated species conservation planning (18). The One Plan Approach to species conservation seeks to unite a wide range of experts from the conservation community: zoo and aquarium professionals, wildlife managers, government agencies, conservation organizations, and local community representatives (18). Together, these stakeholders can establish effective actions across the conservation continuum to serve both *in situ* and *ex situ* populations, including the occasional transfer of individuals between them as founder or reinforcement genetic stock (19). Although the initial focus on ARTs was to address *ex situ* management needs, they will be instrumental in introducing novel genetics via frozen gametes or embryos without the need to remove individuals from the wild (Fig.1).

Prior to the official launch of the One Plan Approach, coordinated planning between *in situ* and *ex situ* stakeholders was occurring in cases where conservation breeding and release were considered essential components of the species recovery program. The red wolf (*Canis rufus*), intentionally extirpated from its range in the southeastern United States to protect the few remaining individuals, has undergone a long and complex restoration that includes a zoo-housed insurance population and free-ranging reintroduced population (20). Similarly, the decline of the Iberian lynx resulted in concerted research efforts to enhance survival of minimal numbers of wild and zoo animals (*Lynx pardinus*) (21). In both species, program managers ensured that living cell samples were cryopreserved for future use. Successful collaborations have sometimes involved multiple nations, such as the Canada-US partnership for the whooping crane (*Grus americana*) recovery program (22). However, population reinforcement with *ex situ*-bred individuals is not the focus for every threatened species. In recent cases, the One Plan Approach has resulted in zoo-based support for in-range community aspects of conservation, such as overcoming human-wildlife conflict, wildlife trafficking, and related issues (23).

Regardless of whether species are kept in the wild or in zoos, some level of human management is involved with animals being confined within enclosed areas restricted by fences, roads, or other human-made barriers on the landscape. When population control is necessary, wildlife managers may have to implement culling or use contraceptive methods (24). Zoo animals do not experience the same pressures as those in the wild, such as predation and disease. Further, advancements in zoo animal husbandry, medicine and welfare have resulted in animals living beyond their natural lifespans, frequently in post-reproductive states (25). While beneficial to individual animals, the advantages of living *ex situ* place a strain on the finite space available. This is particularly relevant to long-living megafauna, such as elephants, rhinoceroses and giraffes. Zoos commonly address density issues by implementing breeding moratoriums, in most cases, with detrimental outcomes long term. A recent assessment of the *ex situ* giraffe population in European zoos following a recommendation to restrict new births demonstrated a shift in population dynamics from actively growing (i.e., reproducing) to ageing (26). This pause in genetic recruitment can threaten the long-term stability of the giraffe program. Currently, public discomfort has prevented euthanasia from being widely accepted as a management tool in zoo populations, even though studies have shown it can lead to more favourable demographic outcomes than contraception (27). While effective messaging and education

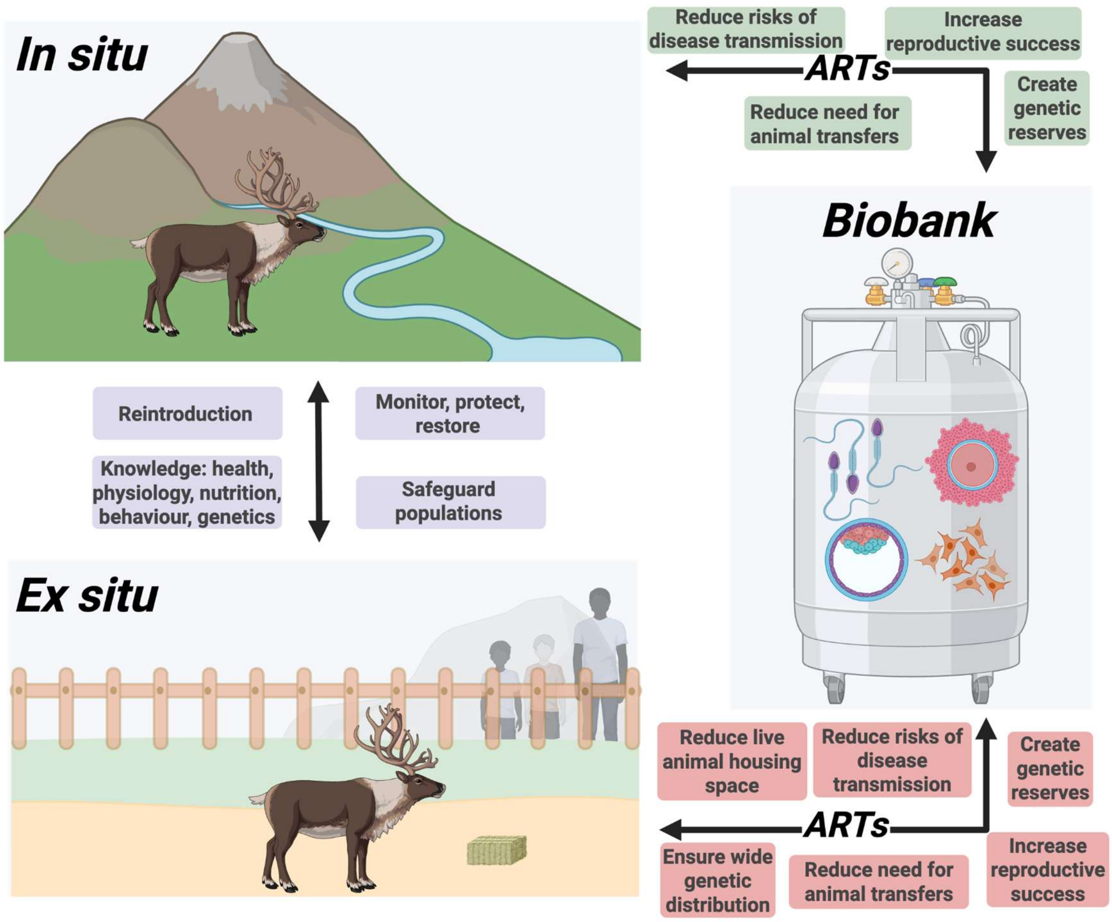

Figure 1. Integrated approach to conservation management. This schematic illustrates how conservation actions are interconnected across *in situ*, *ex situ*, and biobank settings using caribou (*Rangifer tarandus*) as an example species. *In situ* efforts focus on monitoring, protecting, and restoring wild populations, while also contributing to knowledge on health, physiology, nutrition, behavior and genetics. *Ex situ* programs help safeguard populations, support reintroduction, and provide opportunities for genetic management. Biobanks serve as a genetic reserve for both *in situ* and *ex situ* populations, enabling the preservation of gametes, embryos, and cells. Assisted reproductive technologies (ARTs) connect all three systems by reducing the need for live animal transfers, minimizing disease transmission risks, increasing reproductive success, and expanding genetic diversity and distribution. Together, these strategies contribute to a comprehensive and resilient conservation framework. Figure created in BioRender.

are required to change the public's negative perception of euthanasia as a genetic management approach, the need to maintain healthy small, closed populations requires space-saving approaches, such as ARTs, to preserve living cells, capture genetic diversity, and minimize its loss over time. Researchers have begun to generate models that emphasize the cost savings associated with the integration of ARTs and biobanking into long-term population management plans for African wild dogs, as well as marsupials and amphibians [reviewed by (28)].

II. Reproductive management using ARTs

The development of ARTs in wildlife conservation represents a progressive journey, marked by continuous innovation and increasing complexity, with each advancement building upon the successes and challenges of previous techniques to enhance species management and conservation outcomes. These technologies include cryopreservation of somatic cells, gametes and embryos, artificial insemination (AI), *in vitro* embryo production (IVP), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), cloning or somatic cell nuclear transfer (SCNT), and stem cell technologies (Fig. 2). Advantages of implementing ARTs include reducing space requirements for housing live animals, eliminating the need for animal transfers, ensuring wide genetic distribution without the risks of disease transmission, increasing reproductive success and overcoming geographical constraints. Significant research across all vertebrate taxa—mammals, birds, reptiles, amphibians, and fishes—has highlighted the potential for ARTs to support genetic management goals for threatened species (29,30).

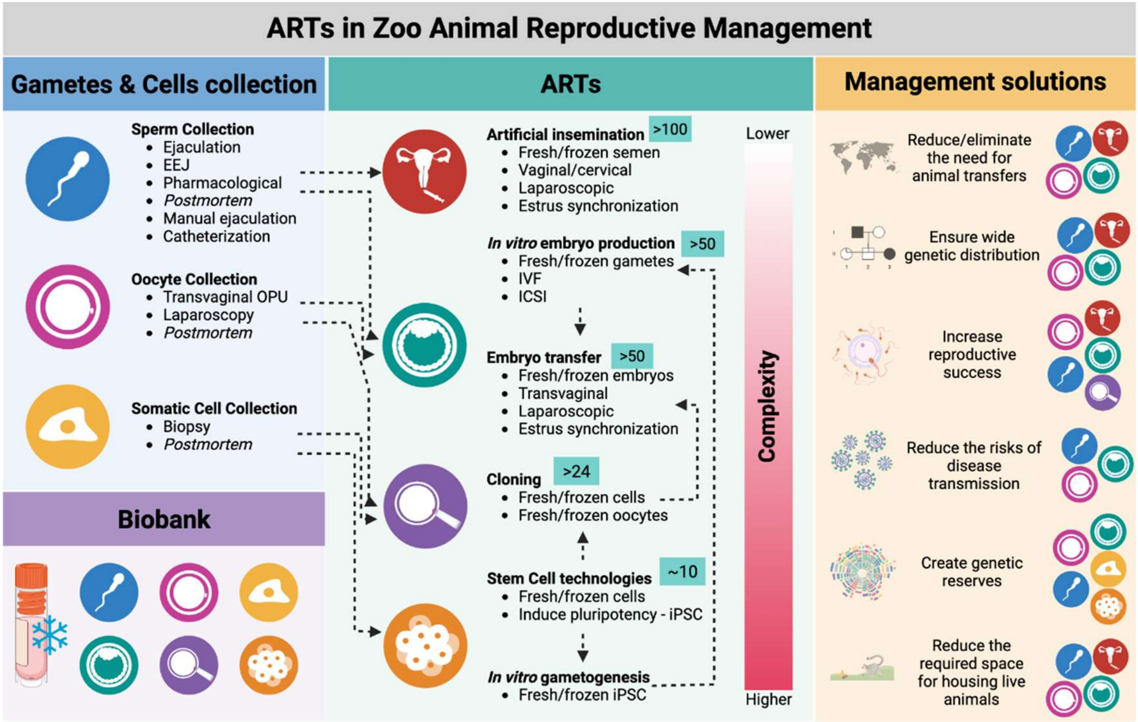

Figure 2. Assisted reproductive technologies (ARTs) in zoo animal reproductive management and their associated conservation benefits. This diagram presents an overview of gamete and somatic cell collection methods, ART procedures, and their associated management solutions in zoo-based conservation. On the left, the sources of biological material include sperm, oocytes, and somatic cells. These materials can be stored in biobanks and used in various ARTs, depicted across a gradient of complexity from artificial insemination (AI), *in vitro* embryo production (IVF, ICSI), and embryo transfer (ET), to more advanced techniques such as cloning, stem cell technologies (iPSCs), and *in vitro* gametogenesis. The number of species in which each ART has been successfully implemented is indicated alongside each method. The right panel outlines key management benefits of ARTs, including reducing the need for animal transfers, increasing reproductive success, ensuring wide genetic distribution, reducing disease transmission risks, creating genetic reserves, and minimizing the space needed for housing live animals. This framework emphasizes how ARTs, when integrated with biobanking, can address conservation challenges while supporting animal health and welfare. Figure created in BioRender.

II.a Successful application of ARTs in wildlife management

Initially, efforts focused on cryopreservation, a foundational technology enabling the long-term storage of genetic materials such as sperm, oocytes, embryos, and somatic cells. Establishing biobanks became crucial, systematically preserving genetic diversity and ensuring the availability of valuable genetic resources for future breeding, reintroduction, and restoration efforts (31). Biobanks today safeguard the genetic heritage of numerous species, including the black-footed ferret (*Mustela nigripes*), giant panda (*Ailuropoda melanoleuca*), cheetah (*Acinonyx jubatus*), wood bison (*Bison bison athabascae*), jaguar (*Panthera onca*), Asian elephant (*Elephas maximus*), among many other species. Despite relative successes in sperm and somatic cells, the cryopreservation of embryos and oocytes remains challenging due to their structural complexity, cellular sensitivity, and the yolk-rich composition of non-mammalian embryos, prompting ongoing research and innovation (32).

AI has emerged as the most widely used ART, primarily due to its minimal invasiveness, relatively straightforward technical requirements, and low operational costs. Consequently, AI has predominantly focused on male genetics, and offspring have been successfully generated in more than 100 species. AI involves collecting sperm from male donors and inseminating females artificially, often after sperm cryopreservation. Methods of sperm collection include manual ejaculation (masturbation, stripping, abdominal massage), electroejaculation, pharmacological stimulation, and *postmortem* retrieval, allowing broader and more versatile use. AI facilitates genetic diversity by enabling genetic exchange across geographically separated individuals without physically moving animals (33,34). Notable AI successes span diverse taxa, such as the blue rock pigeon (*Columba livia*), Swinhoe's pheasant (*Lophura swinhoii*), sandhill crane (*Grus canadensis*), Magellanic penguin (*Spheniscus magellanicus*), fallow deer (*Dama dama*), Eld's deer (*Cervus eldi thamin*), reindeer (*Rangifer tarandus*), banteng (*Bos javanicus*), scimitar-horned oryx (*Oryx dammah*), blackbuck (*Antilope cervicapra*), killer whale (*Orcinus orca*), bottlenose dolphin (*Tursiops truncatus*), African elephant (*Loxodonta africana*), greater one-horned rhinoceros (*Rhinoceros unicornis*), gray wolf (*Canis lupus*), red wolf (*Canis rufus*), clouded leopard (*Neofelis nebulosa*), cheetah (*Acinonyx jubatus*), lion (*Panthera leo*), among others. However, as a routine management tool, AI has only been implemented in a few species such as the giant panda, black-footed ferret, Wyoming toad (*Anaxyrus baxteri*), and Asian elephant (35,36).

IVP further advanced reproductive management by enabling controlled fertilization of oocytes in a laboratory setting, followed by embryo transfer into surrogate females. IVP's significant contribution to genetic management is the ability to preserve female genetics. However, the complexity of IVP necessitates specialized facilities, advanced technical expertise, and substantial research investment. Moreover, oocyte collection presents significant limitations based on species anatomy, achievable through minimally invasive transvaginal ovum pick-up (OPU), which has been employed in wood bison and Przewalski's horse (*Equus ferus przewalskii*), laparoscopic methods for felids and canids, or *postmortem* retrieval in specific scenarios (37–39). Hormonal manipulation required for optimal oocyte retrieval timing introduces physiological and welfare considerations. Therefore, while *postmortem* oocyte retrieval simplifies many logistical and physiological constraints, it restricts applicability in live population management. To address specific reproductive challenges, ICSI, which involves the injection of a single sperm into an oocyte, emerged as an advanced technique. ICSI is valuable for species with compromised sperm quality or minimal sperm availability, proving instrumental in species such as northern white rhinoceros (*Ceratotherium simum cottoni*), and several endangered felids (40,41). Successful IVP applications have been documented in approximately 50 wildlife species, including wood bison, Eld's deer (*Rucervus eldii*), Bengal tiger (*Panthera tigris*), cheetah, clouded leopard (*Neofelis nebulosa*), and amphibians such as the Puerto Rican crested toad (*Peltophryne lemur*), yet routine implementation in conservation remains limited (29,42–44).

Transfers of both *in vivo* derived and IVP embryos for implantation into recipient females have enabled the use of less genetically valuable animals, and even surrogates from closely related or domesticated species. This can enhance reproductive management options, especially for species that are challenging to breed naturally. Cross-species ET successes include Przewalski's horse into domestic mares, bongo antelope (*Tragelaphus eurycerus isaaci*) into eland antelopes (*Taurotragus oryx*), gaur (*Bos gaurus*) into domestic cattle, and southern white rhinoceros hybrid (*Ceratotherium simum*; oocytes fertilized with sperm from northern white rhino) into southern white rhino surrogates, and (41,45–47). Although ET has shown promise, successful application requires extensive research into reproductive biology and estrus synchronization protocols for recipient management.

Cloning via SCNT introduced a revolutionary genetic rescue method with the ability to create offspring from preserved somatic cells. SCNT involves transferring genetic material from somatic cells into enucleated oocytes to produce viable embryos. While often associated with genetic duplication, cloning also holds promise for enhancing genetic variability in managed populations. By accessing historical or underrepresented genotypes stored in biobanked cell lines, particularly from long-deceased founders, SCNT enables the reintroduction of lost alleles and the expansion of genetic diversity within shrinking gene pools (48). Cloning has produced offspring in more than 24 domestic and wildlife species, demonstrating its potential for genetic rescue (48,49). Examples include the European mouflon sheep (*Ovis orientalis musimon*) and gaur (*Bos gaurus*) cloned in 2001, the banteng (*Bos javanicus*) cloned in 2003, the gray wolf cloned in 2005, and the Przewalski's horse cloned in 2020. A particularly significant milestone occurred in 2020 with the birth of a cloned black-footed ferret, Elizabeth Ann, created from preserved cells of a female that died in 1988, introducing valuable genetic diversity into the existing population (50).

Lastly, stem cell technologies, particularly induced pluripotent stem cells (iPSCs), offer innovative conservation strategies (51,52). iPSCs, can be derived from somatic cells and provide a renewable resource to improve the success of SCNT or with potential to generate germ cells *in vitro* (53). Current research includes pioneering efforts with the drill (*Mandrillus leucophaeus*), snow leopard (*Panthera uncia*), prairie vole (*Microtus ochrogaster*), northern white rhinoceros, endangered Southeast Asian primates: celebes crested macaque (*Macaca nigra*), lar gibbon (*Hylobates lar*), and siamang (*Symphalangus syndactylus*), and endangered Japanese birds: Okinawa rail (*Gallirallus okinawae*), Japanese ptarmigan (*Lagopus muta japonica*), and Blakiston's fish owl (*Bubo blakistoni*). These successes highlight unprecedented opportunities to expand the conservation 'toolbox' (54–58). Although they show promise for future conservation planning, practical implementation remains limited due to challenges in species-specific differentiation protocols, genetic stability, and ethical considerations.

II.b Challenges with ARTs

Despite their success, ARTs face several overarching challenges that impede broader implementation in zoo population management, and ultimately, wildlife conservation. A major foundational barrier is the widespread lack of basic reproductive physiology knowledge for many wildlife species. Critical gaps exist in our understanding of reproductive cycles, gamete biology, and species-specific responses to hormone treatments (42,59). Without this baseline knowledge, the development of effective ART protocols remains slow and highly variable across taxa, especially non-mammalian species. This fundamental deficit compounds the challenge of transitioning technologies from experimental research to practical applications, as it impairs the ability to reliably design, test, and refine ART approaches for use in the field or within zoological settings.

Another overarching challenge lies in the complex logistics associated with transporting temperature-sensitive gametes and embryos from field sites to laboratory facilities. Maintaining viability during transit requires precise control of environmental parameters such as temperature, pH, and CO_2/O_2 tension (60). Sperm, which are generally more robust and easier to handle, can often be collected and cryopreserved in the field with minimal equipment, making them more amenable to remote collection protocols. In contrast, oocytes are significantly more fragile and require immediate handling under tightly controlled conditions. Their large size, high lipid content, and susceptibility to temperature fluctuations make them less tolerant to suboptimal conditions during transport (61). This disparity often necessitates field-based interventions, such as initiating *in vitro* maturation on-site or applying temporary arrest strategies using media supplements or temperature modulation, to stabilize oocyte quality during long-distance transport (61). These approaches require portable equipment, sterile conditions, and trained personnel—resources that are often lacking in remote areas. The larger the distance between field collection site and laboratory facility, the greater the risk of gamete degradation, which can severely impact downstream ART outcomes. As a result, developing field-friendly techniques and transport protocols remains a major priority for improving ART feasibility in conservation efforts.

Additionally, the development and refinement of techniques are often hindered by limited access to animals, especially those housed in different zoos, conservation institutions, or *in situ* locations around the world. The geographic dispersion of individuals complicates coordinated reproductive studies, reduces sample sizes for testing protocols, and restricts opportunities for repeat procedures essential for validating ART success (42). A significant obstacle lies in the under-representation of female genetic material in biobanks. Collecting and handling female gametes involves considerable anatomical and physiological challenges. Oocyte retrieval typically requires invasive procedures and specialized handling techniques, restricting their collection in field conditions compared to the relative ease of sperm collection. Moreover, effective cryopreservation protocols for oocytes or embryos remain limited, particularly outside mammalian species. Egg-laying taxa, such as birds, reptiles, amphibians, and fishes, face additional difficulties because yolked eggs are challenging to cryopreserve without compromising viability (62).

High operational costs add further constraints to widespread adoption, especially for embryo-based techniques, which require hormone synchronization, surgical procedures, and often several rounds of attempts. In contrast, sperm-based methods, such as AI, are more affordable, less invasive, and logistically more feasible, accounting for their broader use. Still, even AI's impact on population management remains limited; of the 5,500 mammalian species, only 62 have ever been successfully propagated through AI, and fewer than 40 with frozen-thawed semen (59). For felids, however, ART is among the most promising, with 15 species propagated by AI and 6 with frozen semen. These advances originate from intensive research on domestic cats (*Felis catus*), which share reproductive similarities with wild felids and serve as effective models (59).

Non-scientific barriers also restrict ART use in global conservation efforts. Regulatory restrictions, political boundaries, and societal indifference hinder international collaboration and biological sample exchange. Furthermore, emerging genetic engineering technologies aimed at 'de-extinction'—such as the widely publicized but scientifically debated efforts to resurrect the dire wolf or the development of the genetically engineered woolly mouse—attract significant public attention, risking diverting resources from more practical and currently applicable techniques that are still not widely adopted. Basic ART must be refined and effectively implemented before these cutting-edge technologies can have meaningful conservation impact. Without addressing these foundations, ART's potential to transform wildlife conservation will remain largely unrealized.

Conclusion

The future of species conservation depends on our ability to manage populations across both wild and human care settings with a unified, genetically informed strategy. Historically, zoo-based conservation efforts focused on the management of small, closed populations using conventional demographic and pedigree-based tools. While these approaches laid the groundwork for structured species management, they are insufficient to ensure the long-term genetic sustainability needed for recovery and reintroduction goals. The current shift toward integrated, global population management emphasizes the importance of connecting *in situ* and *ex situ* populations to maintain genetic health across the conservation continuum. This requires incorporating genetic contributions from both *in situ* and *ex situ* individuals, alongside the development of infrastructure and tools to support these efforts—most notably biobanks and ARTs. These innovations provide critical means to preserve, mobilize, and manage genetic diversity across spatial and temporal barriers.

Despite the significant potential of ARTs, their application in wildlife conservation remains limited to a small number of species. Although decades of research have yielded promising results, widespread implementation is hindered by species-specific biological constraints, logistical challenges, and limited operational capacity in the field. Addressing these challenges will require the development of standardized, reproducible protocols adaptable across taxa, along with strategic investment in infrastructure, training, and international collaboration.

At the same time, growing public and scientific interest in emerging technologies, such as genetic engineering and 'de-extinction', has led to significant attention—and significant funding—being directed toward speculative approaches such as the resurrection of the dire wolf or the creation of genetically modified analogs (e.g., woolly mouse) (63). While these technologies may hold future potential, they risk diverting attention from foundational reproductive tools that are urgently needed and already proven effective (64). It is imperative that conservation planning prioritize the refinement and integration of basic ARTs and biobanking before such advanced technologies can be realistically or ethically applied.

As we confront escalating biodiversity loss and environmental instability, the integration of biobanking and ARTs into conservation planning will be indispensable. Moving forward, advancing these tools from niche applications to scalable solutions will be critical for meeting the long-term goals of global species recovery and resilience.

References

Living Planet Report 2024 – A System in Peril. Gland, Switzerland: WWF; 2024.

Richardson K, Steffen W, Lucht W, Bendtsen J, Cornell SE, Donges JF, et al. Earth beyond six of nine planetary boundaries. Sci Adv. 2023 Sep 15;9(37):eadh2458.

Conference of the parties to the Convention on Biological Diversity: Decision adopted by the Conference of the Parties to the Convention on Biological Diversity [Internet]. Montreal, Canada: UN Environment Programme; 2022 Dec. Report No.: CBD/COP/DEC/15/4. Available from: https://www.cbd.int/doc/decisions/cop-15/cop-15-dec-04-en.pdf

Position statement on the role of botanic gardens, aquariums, and zoos in species conservation. [Internet]. Gland, Switzerland: IUCN Species Survival Comission; 2023. Available from: https://iucn.org/sites/default/files/2023-10/2023-position-statement-on-the-role-of-botanic-gardens-aquariums-and-zoos-in-species-conservation.pdf

Frankham R. Genetics and extinction. Biological Conservation. 2005 Nov;126(2):131–40.

Caughley G. Directions in Conservation Biology. The Journal of Animal Ecology. 1994 Apr;63(2):215.

Soulé M, Gilpin M, Conway W, Foose T. The millenium ark: How long a voyage, how many staterooms, how many passengers? Zoo Biology. 1986 Jan;5(2):101–13.

Lees CM, Wilcken J. Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. International Zoo Yearbook. 2009 Jan;43(1):6–18.

Foose TJ, Ballou JD. Management of small populations. International Zoo Yearbook. 1988 Jan;27(1):26–41.

Elsbeth McPhee M. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation. 2004 Jan;115(1):71–7.

Christie MR, Marine ML, French RA, Blouin MS. Genetic adaptation to captivity can occur in a single generation. Proc Natl Acad Sci USA. 2012 Jan 3;109(1):238–42.
Montgomery ME, Ballou JD, Nurthen RK, England PR, Briscoe DA, Frankham R. Minimizing kinship in captive breeding programs. Zoo Biol. 1997;16(5):377–89.
Moreno E, Cervantes I, Gutiérrez JP, Fernández I, Goyache F. Analysing the pedigree to identify undesirable losses of genetic diversity and to prioritize management decisions in captive breeding: a case study. Heredity. 2024 Dec;133(6):400–9.
Che-Castaldo J, Gray SM, Rodriguez-Clark KM, Schad Eebes K, Faust LJ. Expected demographic and genetic declines not found in most zoo and aquarium populations. Frontiers in Ecol & Environ. 2021 Oct;19(8):435–42.
Irizarry KJL, Bryant D, Kalish J, Eng C, Schmidt PL, Barrett G, et al. Integrating Genomic Data Sets for Knowledge Discovery: An Informed Approach to Management of Captive Endangered Species. International Journal of Genomics. 2016;2016:1–12.
Humble E, Dobrynin P, Senn H, Chuven J, Scott AF, Mohr DW, et al. Chromosomal-level genome assembly of the scimitar-horned oryx: Insights into diversity and demography of a species extinct in the wild. Molecular Ecology Resources. 2020 Nov;20(6):1668–81.
Speak SA, Birley T, Bortoluzzi C, Clark MD, Percival-Alwyn L, Morales HE, et al. Genomics-informed captive breeding can reduce inbreeding depression and the genetic load in zoo populations. Molecular Ecology Resources. 2024 Oct;24(7):e13967.
Gusset M. Zoos and Aquariums Committing to Integrated Species Conservation. In: The Routledge Handbook of Animal Ethics [Internet]. 1st ed. New York: Routledge; 2019 [cited 2025 Apr 16]. p. 57–366. Available from: https://www.taylorfrancis.com/books/9781351602372
CPSG. One Plan Approach to Species Conservation Planning [Internet]. Available from: https://www.cpsg.org/our-work/our-approach/one-plan-approach
Hinton J, Chamberlain M, Rabon D. Red Wolf (Canis rufus) Recovery: A Review with Suggestions for Future Research. Animals. 2013 Aug 13;3(3):722–44.
Vargas A, Sánchez I, Martínez F, Rivas A, Godoy JA, Roldán E, et al. The Iberian lynx *Lynx pardinus* Conservation Breeding Program. International Zoo Yearbook. 2008 Apr;42(1):190–8.
Barrett C, Stehn T. A retrospective of whooping cranes in captivity. In: Hartup, Barry K, ed, Proceedings of the Eleventh North American Crane Workshop. Wisconsin Dells; 2008. p. 166–79.
Serota MW, Barker KJ, Gigliotti LC, Maher SML, Shawler AL, Zuckerman GR, et al. Incorporating human dimensions is associated with better wildlife translocation outcomes. Nat Commun. 2023 Apr 25;14(1):2119.
Massei G. Fertility Control for Wildlife: A European Perspective. Animals. 2023 Jan 27;13(3):428.
Tidière M, Gaillard JM, Berger V, Müller DWH, Bingaman Lackey L, Gimenez O, et al. Comparative analyses of longevity and senescence reveal variable survival benefits of living in zoos across mammals. Sci Rep. 2016 Nov 7;6(1):36361.
Scherer L, Bingaman Lackey L, Hahn-Klimroth M, Müller D, Roller M, Bertelsen M, et al. Assessing zoo giraffe survivorship: Methodological aspects, historical improvement and a rapid demographic shift. Journal of Zoo and Aquarium Research. 2024 Apr 30;12(2):88–101.
Clauss M, Roller M, Bertelsen MF, Rudolf Von Rohr C, Müller DWH, Schiffmann C, et al. Zoos must embrace animal death for education and conservation. Proc Natl Acad Sci USA. 2025 Jan 7;122(1):e2414565121.
Paris DBBP, Riddell P, Joone C, De La Rey M, Ganswindt A, Paris MCJ. Cold dogs: Sperm freezing, artificial insemination & non-invasive monitoring tools to facilitate a hybrid conservation management approach for endangered African wild dogs. Theriogenology Wild. 2024;4:100073.
Mastromonaco GF, Songsasen N. Reproductive technologies for the conservation of wildlife and endangered species. In: Reproductive Technologies in Animals [Internet]. Elsevier; 2020 [cited 2025 Apr 10]. p. 99–117. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128171073000072
Pukazhenthi BS, Wildt DE. Which reproductive technologies are most relevant to studying, managing and conserving wildlife? Reprod Fertil Dev. 2004;16(2):33.
Chen DM, Mastromonaco GF. The Evolution of Conservation Biobanking: A Literature Review and Analysis of Terminology, Taxa, Location, and Strategy of Wildlife Biobanks Over Time. Biopreservation and Biobanking. 2025 Feb 12;bio.2024.0151.
Comizzoli P, Songsasen N, Hagedorn M, Wildt DE. Comparative cryobiological traits and requirements for gametes and gonadal tissues collected from wildlife species. Theriogenology. 2012;78(8):1666–81.

Wildt DE, Comizzoli P, Pukazhenthi B, Songsasen N. Lessons from biodiversity - The value of nontraditional species to advance reproductive science, conservation, and human health. Molecular Reproduction and Development. 2010;77(5):397–409.
Howard JG, Lynch C, Santymire RM, Marinari PE, Wildt DE. Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. Animal Conservation. 2016;19(2):102–11.
Kouba AJ, Vance CK, Willis EL. Artificial fertilization for amphibian conservation: Current knowledge and future considerations. Theriogenology. 2009 Jan;71(1):214–27.
Roth TL, Swanson WF. From petri dishes to politics – a multi-pronged approach is essential for saving endangered species. Nat Commun. 2018 Jul 4;9(1):2588.
Novak BJ, Ryder OA, Houck ML, Walker K, Russell L, Russell B, et al. Endangered Przewalski's Horse, Equus przewalskii, Cloned from Historically Cryopreserved Cells. Animals (Basel). 2025 Feb 20;15(5):613.
Cervantes MP, Palomino JM, Anzar M, Mapletoft RJ, Mastromonaco GF, Adams GP. In vitro-production of embryos using immature oocytes collected transvaginally from superstimulated wood bison (Bison bison athabascae). Theriogenology. 2017;92:103–10.
Jorge-Neto PN, Requena LA, de Araújo GR, Traldi A de S, Luczinski TC, de Deco-Souza T, et al. Efficient recovery of in vivo mature and immature oocytes from jaguars (Panthera onca) and pumas (Puma concolor) by Laparoscopic Ovum Pick-Up (LOPU). Theriogenology Wild. 2023 Jan 1;3:100042.
H**ildebrandt TB, Holtze S, Colleoni S, Hermes R, Stejskal J, Lekolool I, et al.** In vitro fertilization program in white rhinoceros. Reproduction. 2023 Sep 20;166(6):383–99.
Summers PM, Shephard AM, Hodges JK, Kydd J, Boyle MS, Allen WR. Successful transfer of the embryos of Przewalski's horses (Equus przewalskii) and Grant's zebra (E. burchelli) to domestic mares (E. caballus). Reproduction. 1987 May 1;80(1):13–20.
Herrick JR. Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. Biology of Reproduction. 2019 May 1;100(5):1158–70.
Burger I, Julien AR, Kouba AJ, Barber D, Counsell KR, Pacheco C, et al. Linking in-situ and ex-situ populations of threatened amphibians through genome banking. Conservat Sci and Prac. 2021 Nov;3(11):e525.
S**illa AJ, Calatayud NE, Trudeau VL.** Amphibian reproductive technologies: approaches and welfare considerations. Cooke S, editor. Conservation Physiology. 2021 Jan 1;9(1):coab011.
Dresser B, Pope CE, Kramer L, Dalhausen R, Maruska E, Thomas W. Birth of bongo antelope (Tragelaphus euryceros) to eland antelope (Tragelaphus oryx) and cryopreservation of bongo antelopes. In Theriogenology; 1985. p. 190.
Hammer CJ, Tyler HD, Loskutoff NM, Armstrong DL, Funk DJ, Lindsey BR, et al. Compromised development of calves ( ) derived from in vitro-generated embryos and transferred interspecifically into domestic cattle ( ). Theriogenology. 2001 Apr;55(7):1447–55.
Korody ML, Hildebrandt TB. Progress Toward Genetic Rescue of the Northern White Rhinoceros (Ceratotherium simum cottoni). Annual Review of Animal Biosciences. 2025 Feb 18;13(1):483–505.
Mastromonaco GF, González-Grajales LA, Filice M, Comizzoli P. Somatic Cells, Stem Cells, and Induced Pluripotent Stem Cells: How Do They Now Contribute to Conservation? In 2014. Available from: http://link.springer.com/10.1007/978-1-4939-0820-2_16
Olsson PO, Jeong YW, Jeong Y, Kang M, Park GB, Choi E, et al. Insights from one thousand cloned dogs. Sci Rep. 2022 Jul 1;12(1):11209.
Wildt DE, Rall WF, Critser JK, Monfort SL, Seal US. Genome Resource Banks. BioScience. 1997;47(10):689–98.
Hutchinson AM, Appeltant R, Burdon T, Bao Q, Bargaje R, Bodnar A, et al. Advancing stem cell technologies for conservation of wildlife biodiversity. Development. 2024 Oct 15;151(20):dev203116.
Marx V. Can stem cells save the animals? Nat Methods. 2025 Jan;22(1):8–12.
Saitou M, Hayashi K. Mammalian in vitro gametogenesis. Science. 2021 Oct;374(6563):eaaz6830.
Hildebrandt TB, Hermes R, Colleoni S, Diecke S, Holtze S, Renfree MB, et al. Embryos and embryonic stem cells from the white rhinoceros. Nat Commun. 2018 Jul 4;9(1):2589.
Friedrich Ben-Nun I, Montague SC, Houck ML, Tran HT, Garitaonandia I, Leonardo TR, et al. Induced pluripotent stem cells from highly endangered species. Nature Methods. 2011;8(10):829–31.
Bao Q, Tay NL, Lim CY, Chua DHH, Kee SK, Choolani M, et al. Integration-free induced pluripotent stem cells from three endangered Southeast Asian non-human primate species. Sci Rep. 2024 Jan 29;14(1):2391.

Manoli DS, Subramanyam D, Carey C, Sudin E, Van Westerhuyzen JA, Bales KL, et al. Generation of Induced Pluripotent Stem Cells from the Prairie Vole. Muotri AR, editor. PLoS ONE. 2012 May 31;7(5):e38119.
Katayama M, Fukuda T, Kaneko T, Nakagawa Y, Tajima A, Naito M, et al. Induced pluripotent stem cells of endangered avian species. Commun Biol. 2022 Oct 24;5(1):1049.
Swanson WF. The challenge of assisted reproduction for conservation of wild felids - A reality check. Theriogenology. 2023 Feb;197:133–8.
Sciorio R, Rinaudo P. Culture conditions in the IVF laboratory: state of the ART and possible new directions. J Assist Reprod Genet. 2023 Nov;40(11):2591–607.
Comizzoli P, Songsasen N, Wildt DE. Protecting and Extending Fertility for Females of Wild and Endangered Mammals. In: Woodruff TK, Zoloth L, Campo-Engelstein L, Rodriguez S, editors. Oncofertility [Internet]. Boston, MA: Springer US; 2010 [cited 2025 Apr 16]. p. 87–100. (Cancer Treatment and Research; vol. 156). Available from: http://link.springer.com/10.1007/978-1-4419-6518-9_7
Hu T, Taylor L, Sherman A, Keambou Tiambo C, Kemp SJ, Whitelaw B, et al. A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells. eLife. 2022 Jan 25;11:e74036.
Kluger J. The Return of the Dire Wolf. Time [Internet]. 2025 Apr 7; Available from: https://time.com/7274542/colossal-dire-wolf/
DeJong D. This is the real problem with the "de-extinction" hype. Toronto Star [Internet]. 2025 Apr 15; Available from: https://www.thestar.com/opinion/contributors/this-is-the-real-problem-with-the-de-extinction-hype/article_b1134f85-e272-4496-9fe8-fac2b133a9a5.html